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Antimicrobial Activity of Rice Starch based Packaging Film Loaded with Zinc Oxide Nanoparticles

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Abstract: In this work ZnO nanoparticles were loaded in rice starch based films using an unique equilibration-cum-hydrothermal approach. The films have been characterized by XRD, SPR and SEM analysis. These films have shown excellent antibacterial action against model bacteria E.coli when investigated qualitatively by zone inhibition method. Films exhibit great potential to be used as packaging films to protect food stuff against microbial contaminants.

Keyword: Antimicrobial activity, Rice starch film, ZnO nanoparticles.

I. INTRODUCTION

Films and coatings prepared from biodegradable materials are increasingly being used in the food packaging industry [1]. Biodegradable polymers can be produced from natural, renewable resources (for example, starch), chemically synthesized from natural sources (for example, poly[lactic acid]), or made from microbiologically produced materials (for example, hydroxybutyrate and hydroxyvalerate)[1][2][3][4][5][6][7] These biopolymers can decompose more readily in the environment than their synthetic polymeric counterparts such as polyethylene (PE), polypropylene (PP), and polystyrene (PS) that arederived from crude oils[8][9][10][11][12][13][14]. Consumer demands for preservative-free, high-quality food products, packaged in materials that create less environmental impact have inspired research into the application of biopolymeric materials. In combination with antimicrobial (AM) packaging systems, biopolymer materials with AM properties are emerging as one of the more promising forms of active packaging systems [15][16][17]. Further development of food packaging materials manufactured from biodegradable polymers such as starch-based materials have the potential to reduce environmental impacts thereby being advantageous over conventional synthetic-based packaging systems [18].

The current research has been focused on the search for new bactericides that can effectively reduce the harmful effects of microorganisms. With the emergence of nanotechnology, the search for effective biocidal agents has been concentrated on the development of nanostructures of coinage metals like silver, copper, zinc and gold[19]**.** Recently, there have been several reports regarding the antimicrobial activity of ZnO nanoparticles [20]**.** It has been reported by [21]**,** on the basis of preliminary growth analysis, that ZnO nanoparticles have higher antibacterial affects on microorganism like S. aureus than other metal oxide nanoparticles.It was observed that nanoZnO showed enhanced antibacterial activity as compared to bulk ZnO which is attributed to the generation of reactive oxygen species (ROS) on the surface of ZnO nanoparticles.ROS are species such as superoxide, hydroxyl radicals etc which are actively involved in damage of bacterial cells. In order to facilitate homogeneous distribution of ZnO nanoparticles within the film matrix,we have developed an unique approach that involves preparation of film from aqueous solution of starch and Zn(II) ions by solvent evaporation method, followed by in-situ precipitation of Zn(II) as $Zn(OH)_2$ within the film matrix, which on hydrothermal treatment yields ZnO nanoparticles loaded starch film.

II. MATERIAL AND METHODS

Material

Rice starch was obtained from Pruthvi's Foods Private limited, Gujarat, India and used as received. Zinc chloride, sodium hydroxide, glycerolwere purchased from,Qualikems Fine ChemicalsPvt. Ltd. New Delhi,India and usedas received. Nutrient agar, agar-agar type I were received from S. D. Fine Chemicals, Mumbai, India.The Milli-pore water (conductivity 0.06–0.10 μS/cm and bacterial count<10CFU/ml) was used throughout the investigations.

Preparation of Rice starch based films loaded with Zinc oxide nano particles.

Plain rice starch based film (PRS) was prepared by microwave induced gelatinization of rice starch followed by solvent evaporation. 2 g of rice starch was added into 30 ml of distilled water at 75°C followed by addition of

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0.2 ml of glycerol. The total volume of colloidal dispersion, so obtained, was made up to 35 ml by addition of appropriate quantity of water. The colloidal dispersion was put in microwave oven (LG, model MS-1947C) and irradiated at 640 Watt for 20 seconds, which yielded an almost transparent solution. The solution was poured into Petri dish and put in an electric oven (Tempstar, India) at 55^{o}C for a period of 24 hrs. Finally the film was pealed off and kept in a dessicator for further use. The ZnO nanoparticles loaded rice starch film was prepared by in situ formation of zincoxide within the rice starch film using the hydrothermal approach. In brief, to 35 ml of the above colloidal dispersion of rice starch in water, a precalculated amount of $ZnCl_2$ was added and the resulting solution was transferred into Teflon coated Petri dishes and kept in an electric oven (Tempstar, India) at 80°C for a period of 12 h.

The film, thus formed, was peeled off and put in a 0.02M solution of sodium hydroxide. After 4 h, the film was taken out and kept in an electric oven at 70°C for complete conversion of $Zn(OH)_2$ into ZnO. Finally, the film was washed with distilled water and dried in a dust free chamber at ambient temperature until it was completely dry. The films were designated as ZONLRS(X) where the number X in parenthesis denotes the concentration of Zn(II) ions (in percent W/V)in starch solution.

III. CHARACTERIZATION OF FILM

XRD analysis
XRD analysis was performed with a Rigaku Miniflex IIdesktop Xray Diffractometer (Japan).

SEM analysis
The morphological features of plain rice starchfilm and ZnO nanoparticles loaded rice starch film were observed using a JEOL JASM-6200 (Japan) Analytical Scanning Electron Microscope.

UV-Visible spectrum analysis/SPR
The UV-Visible spectrum of the nano ZnO dispersed in distilled water was recorded in a UV-Visible spectrophotometer (Shimadzu 6300) in the range of 300–550 nm. The zinc oxide (ZnO) nanoparticles were prepared by the wet chemical method using zinc nitrate and sodium hydroxide as precursors. Zinc nitrate, 14.874 g (0.1 mol), was dissolved in 500 ml of distilled water under vigorous stirring to ensure complete dissolution.

After complete dissolution, 0.2 mol of sodium hydroxide solution was added under constant stirring, drop by drop touching the walls of the vessel. The reaction was allowed to proceed for 2 h after complete addition of sodium hydroxide. The solution was then allowed to settle overnight and supernatant was discarded carefully. The remaining solution was centrifuged at 200 rpm for 10 min and the supernatant was discarded. The residual mass was dried at 80°C for overnight. During drying, complete conversion of $Zn(OH)_2$ into ZnO took place. A 0.2% (w/v) solution was used to record UV-Vis spectrum.

Antimicrobial studies
The biocidal action of ZnO nanoparticles loaded rice starch films(ZONLRS) was investigated in qualitative manner, by the zone inhibition method [22] respectively, with E. coli as the model bacteria. One hundred micro liters of the inoculums solution was added to 20 ml of the appropriate softagar, which was overlaid on to Petri dishes. Square shaped films were cut from the test films and placed on the bacterial lawns.

The Petri dishes were incubated for 48 hrs at 37^{o}C in the aerobic incubation chamber. The Petri dishes were examinedvisually for zones of inhibition around the square shaped films , and the size of the zone diameter was measured at two cross sectional points and the average was taken as the inhibition zone. The same procedure was followed with plain rice starch film.

IV. RESULTS AND DISCUSSION

Characterization of ZONLRS film
UV –Visible /spectral analysis:
The surface plasmon resonance (SPR) is a characteristic of metal nanoparticles. The room temperature UV-Vis absorbance spectrum for the ZnO particles has been shown in Figure 1. The sharp absorbance peak, located at about 362nm, corresponds to the band gap of 3.42 eV. This is almost in accordance with the value of bulk ZnO [23], thus suggesting excellent crystal quality of the ZnO nanoparticles.

Therefore, no blue shift was observed in UV-Vis spectrum, revealing that nanoscale ZnO particles obtained are not small enough to show quantum confinement related effects. In Surface Plasmon Spectrum for ZnO nanoparticles an asymmetric tail can also be found on higher wavelength of the peak, induced by light scattering[4].

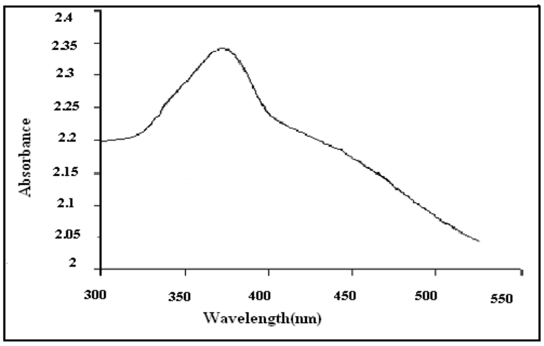

Fig.1 Surface Plasmon Spectrum for ZnO nanoparticles

XRD analysis

Figure 2 shows the X-ray diffraction pattern of ZnO nanoparticles loaded rice starch film. The peaks, observed at 2θ values of 32.7^o ,36.2^o ,49.5^o ,62.8^o and 69.1^o,correspond to the reflections at (100), (101), (102),(103), (112) planes respectively [JCPDS76-0704].Almost similar value have also been reported by [24].

The above XRD pattern also consists of some additional sharp peaks showing presence of crystalline starch matrix .The diffraction peaks observed at 5.6^o, 19^{o}and 26^o indicate that crystalline type of rice starch is intermediate to that of Type A and Type B starches [25].

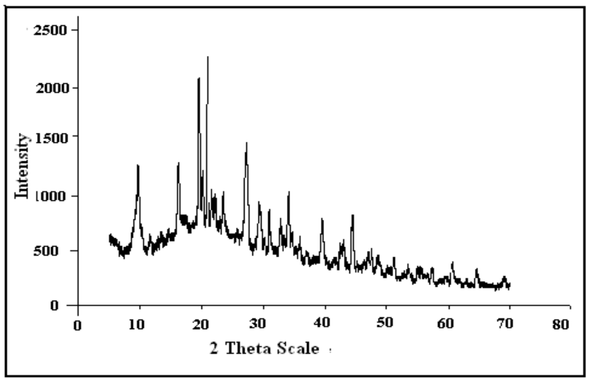

Fig.2 XRD of ZnO nanoparticles loaded rice starch film

SEM analysis

Scanning electron microscopy (SEM) is an effective tool to study the surface morphology of materials. Figure 3 (a) and (b) give a comparative depiction of the SEM images of plain rice starch (PRS)film and zinc oxide nano particles loaded rice starch film (ZONLRS) respectively. It is quite evident fromFigure 3(a) that PRS film exhibits smooth surface while the surface of ZONLRS film , as shown in Figure 3(b),demonstrates crystalline structure of ZnO nanoparticles in an almost uniformly distributed manner. The average size of the ZnO nanoparticles was found to be approximately 977 nm which, in fact, lies far away from the prescribed size of nanoparticles [26].

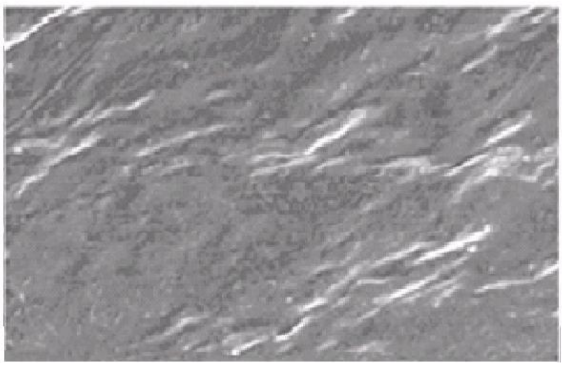

Fig.3 (a) SEM image of Plain rice starch film

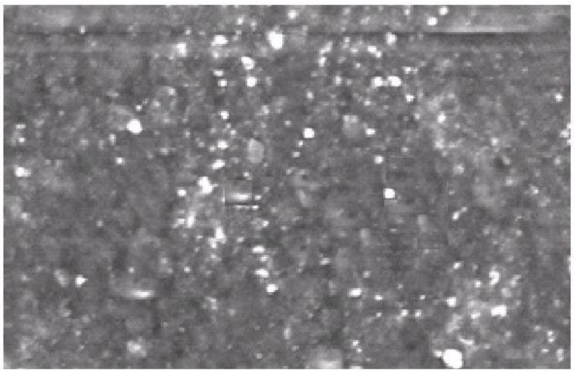

Fig.3 (b) SEM image of ZnO nano particles loaded rice starch film

ANTIBACTERIAL INVESTIGATIONS

The antibacterial action of ZONLRS film was tested against E-Coli as model bacteria, taking plain rice starch (PRS) film as control. The results of investigations have been well depicted in Figure 4. It is clear from Figure 4 that there is dense population of bacterial cells in the petriplate supplemented with PRS film while a clear zone of inhibition appears around the piece of ZONLRS film in the petri plate as shown in Figure 4. films. Therefore, it is clear that zinc oxide nanoparticles loaded rice starch film has the potential to inhibit bacterial colony

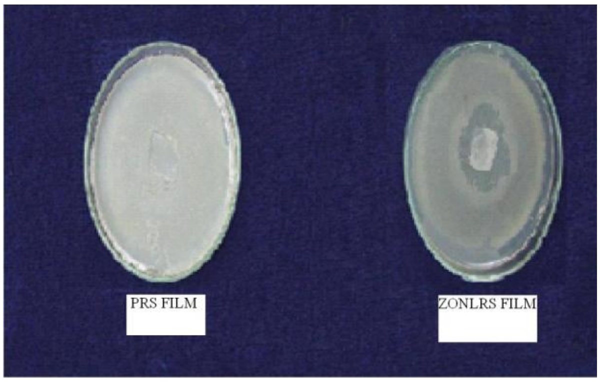

Fig. 4Antimicrobial activity in (A) PRS film (control) (B) ZONLRS film (6% ZnO nano particles)

V. CONCLUSION

From the above study it may be concluded that rice starch based films can be used as packaging film to restrict bacterial growth in food products.

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